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BLADDER CANCER

Combining epigenetic and immune checkpoint inhibitors in bladder cancer

At the end of treatment, 75% of mice treated with anti-PD-L1 alone but only <30% of those treated with anti-PD-L1 plus CM-272 still had tumours and metastases.

A new study, published in *Nature Medicine*, identifies the histone methyltransferase G9a (also known as EHMT2) as a new target for bladder cancer treatment. Simultaneous inhibition of G9a and programmed cell death 1 ligand 1 (PD-L1) resulted in significant and sustained responses in a mouse model of metastatic bladder cancer. Furthermore, the researchers also found that immunotherapy resistance in patients is associated with increased G9a levels, indicating the clinical relevance and utility of this combination treatment.

The study is a collaborative effort of researchers based in Pamplona and Madrid, Spain. “Over the past 10 years, we have found that epigenetic mechanisms play crucial roles in bladder cancer recurrence and progression and that mechanisms under the control of the histone-lysine *N*-methyltransferase EZH2 are essential in these processes, pointing to a potential use of EZH2 as a therapeutic target; however, the use of EZH2 inhibitors proved technically difficult,” senior author Jesus Paramio tells *Nature Reviews Urology*. “The group from Pamplona is interested in epigenetics as a target for various malignancies and has developed inhibitors of several epigenetic and chromatin remodelling proteins, including CM-272, which targets G9a and DNA methyltransferases (DNMTs).”

Using data from patients with non-muscle-invasive bladder cancer, the researchers observed that G9a levels were significantly higher in

advanced-stage, high-grade and recurrent or progressive tumours than in normal bladder tissue. Furthermore, high G9a expression was associated with significantly increased disease recurrence, indicating that G9a might be a treatment target.

“Remarkably, when tested in different tumour cell lines, CM-272 showed one of the highest activities against bladder cancer cells,” says Paramio. “As G9a physically interacts with EZH2 and can affect its activity, we decided to explore its activity alone or in combination with cisplatin.” Combination treatment with CM-272 plus cisplatin resulted in significantly decreased tumour growth in xenograft models. In a metastatic bladder cancer model with urothelial-cell-specific knockout of *Pten*, *Trp53* and *Rb1* in *Rb1*-deficient mice, treatment with CM-272 or CM-272 plus cisplatin resulted in a significant reduction of tumour and metastatic burden. Transcriptome analyses to characterize the mechanism of action in vivo indicated upregulation of several inflammatory response genes, allograft rejection genes, chemoattractants and activators of T cell and natural killer cell ligands and receptors, and downregulation of various immunoregulatory cytokines. The team also observed infiltration of CD8⁺ T cells and natural killer cells and reduced numbers of CD163⁺ cells.

“When analysing the mechanism of action, we observed a potential reactivation of the immune system

against the tumours,” summarizes Paramio. “We thus studied the effects of the combination of CM-272 and an immune checkpoint inhibitor.”

Treatment of the transgenic mice with an anti-PD-L1 compound alone or combined with CM-272, or with CM-272 plus cisplatin, showed that, at mid-treatment, tumour burden was similar between groups but those treated with CM-272 plus anti-PD-L1 had extensive immune cell infiltrations. At the end of treatment, 75% of mice treated with anti-PD-L1 alone but only <30% of those treated with anti-PD-L1 plus CM-272 still had tumours and metastases. When the mice treated with the combination were maintained for a further 4 weeks, tumours and metastases did not regrow.

The team also investigated the role of G9a and EZH2 in the immune checkpoint inhibitor response of patients with bladder cancer. Multivariate analysis that included various pathological parameters showed that G9a expression was significantly associated with resistance to immunotherapy, indicating the possibility to use G9a inhibitors to increase the effectiveness and utility of immunotherapy in patients with bladder cancer.

“We are now pursuing further basic and translational research on the epigenetic mechanisms in bladder cancer, also including other inhibitors and immunotherapies,” concludes Paramio. “The most important aspect for the future is the implementation of this research in the clinic: trying to start clinical trials with this or more advanced inhibitors alone or in combination with immunotherapies.”

Clemens Thoma

ORIGINAL ARTICLE Segovia, C. et al. Inhibition of a G9a/DNMT network triggers immune-mediated bladder cancer regression. *Nat. Med.* **25**, 1073–1081 (2019)

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